REVIEW ARTICLE

Current and future prospective of liposomes as drug delivery vehicles for the effective treatment of cancer

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Abstract

Liposomes, as the leading drug delivery system, have played a significant role in the formulation of anticancer drug to improve therapeutic effect. This system improves the pharmacokinetic and pharmacodynamic profiles of the therapeutic payload, promote controlled and sustained release of anticancer drugs, and exhibit very less systemic toxicity as compared to the free anticancer drug. The mechanism giving rise to therapeutic advantages of liposomes such as the ability of long-circulating liposomes to preferentially accumulate at disease sites such as tumors, site of infection, and site of inflammation. In the studies, liposomal anthracyclines have shown highly efficient drug encapsulation, resulting in significant anticancer activity with reduced cardiotoxicity. There are several methods for liposomes preparation based on lipid drug interaction and liposomes disposition mechanism including the incubation of rapid clearance of liposomes by controlling particle size and surface hydration. The liposomes are characterized with respect to physical, chemical, and biological parameters. This review discusses the recent advances in the preparation methods of liposome for the treatment of cancer.

Key words: Characterization, drug carrier, liposome, phospholipids, targeted site

INTRODUCTION

Nowadays, liposomes can be frequently used to recover existing cancer treatment due to their rise in the solubility of water-insoluble antitumor drugs. The long-circulating liposomes also act to decrease the mononuclear phagocyte system's uptake due to a passive directing toward cancer.[1,2] These approaches reduce the degradation of drug and drug inactivation on administration, as well as an increase in the bioavailability of antitumor drug and the fraction of delivered drug within the cancer area, thus increasing in efficacy of delivered drug and minimizing drug toxicity and side effect too.

DEFINITION, STRUCTURE, AND CLASSIFICATION OF LIPOSOMES

"Liposomes are spherical-shaped vesicles composed of one or more lipid bilayers, involving an aqueous compartment" as shown in Figure 1. These are molded spontaneously; first, the lipids are dispersed in an aqueous medium by continuous stirring, which produce vesicles may in size range from nanometers to microns in breadth.[3] The lipid molecules consist head moiety which are attracted to hydrophilic molecules and shape themselves in such a way as to point toward the aqueous zone, whereas the lipophilic tails are repelled by the water molecules and point in the opposite way.

The hydrophilic groups of the inner layer point in the side of the intravesicular fluid, with the tails pointing away from it. As such, the lipophilic tails of one layer point toward the hydrocarbon tails of the outermost layer, in turn forming the normal bilipid membrane.[4-6]

Phospholipids and sphingolipids are the choice of lipids that are mostly used in the preparation of liposomes. Both

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of them are biodegradable and biocompatible in nature. These lipids are either originated from natural or synthetic sources. In contrast, used lipids are cylindrical molecular shape, to organize into stable bilayers in aqueous solutions. The phosphatidylcholines are the mostly used due to their stability and their ability to against in pH changes or changes in concentrations of salt in the product or in biological environment.[7]

Liposomes are distinguished in terms of their size (small, intermediate, or large), bilayers count (unilamellar and multilamellar), composition, and mechanism of delivery of drug. Small unilamellar vesicles comprise of a single lipid bilayer with an average diameter ranging from 25 to 75 nm. Large unilamellar vesicles also comprise of a single lipid bilayer and are >75 nm, on the other hand, multilamellar vesicles (MLVs) are composed of many concentric lipid bilayers and measure of 1-5 μm[5,8] [Figure 2].

As regards the arrangement and mechanism of delivery of drug, the liposomes can be categorized as conventional liposomes, long-circulating liposomes, polymorphic liposomes (pH-sensitive, thermo-sensitive, and cationic liposomes), and decorated liposomes (surface-modified liposomes and immune liposomes) as shown in Figure 3.

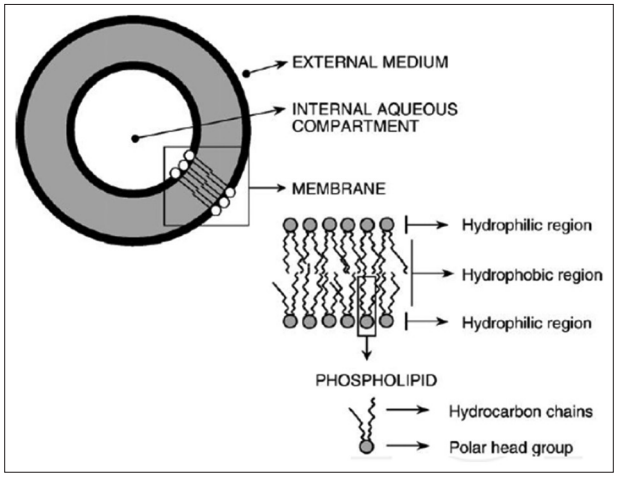

Figure 1: Structure and composition of liposomes[3]

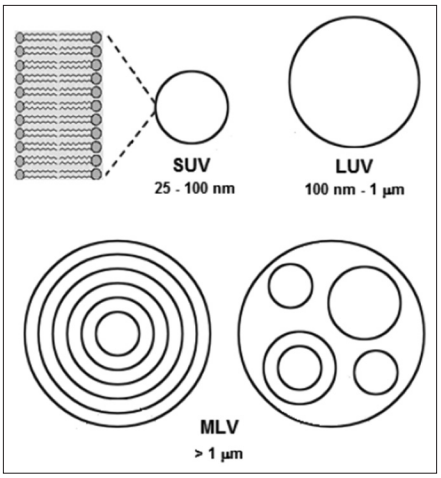

Figure 2: Categorization of liposomes[6]

PREPARATION METHODS FOR LIPOSOMES

As abovementioned, liposomes are spontaneously molded when phospholipids are hydrated. Additional steps are habitually essential to alter the size spreading and lamellarity of the liposomes. Liposome preparation includes three main steps:

1. Formation of vesicle
2. Decrease in vesicle size decrease and
3. Purification of liposomes.

Numerous preparation approaches have been recognized based on the scale of the manufacture and other respects, such as drug encapsulation effectiveness, the drug's physicochemical features, and the administration route [Table 1].

The mostly used methods for preparation of liposomes are lipid hydration and the replacement of organic solvents by an aqueous media (reverse-phase evaporation and organic solvent injection). The lipid hydration trailed by vortexing or

Table 1: Methods of liposomes preparation

Vesicles formation	Liposomes' types
Lipid hydration method trailed by vortexing or stirring	MLV
Reverse-phase evaporation method	MLV, LUV
Organic solvent injection method	MLV, LUV, SUV
Freeze-thawing method	MLV, LUV
pH gradient method	LUV, SUV
Dehydration-rehydration technology	MLV
Detergent dialysis technology	MLV, LUV
Vesicle size reduction	
Extrusion through membranes	LUV, SUV
High-pressure homogenization technology	LUV, SUV
Micro fluidization method	SUV
Sonication method	SUV
Purification	
Centrifugation technology	
Dialysis technology	
Column chromatography separation technology	
Ultrafiltration technology	

SUV: Small unilamellar vesicle, LUV: Large unilamellar vesicle, MLV: Multilamellar vesicle

stirring, known as "Bangham's method," method comprised of dissolving the lipids in an organic solvent, such as chloroform or methanol. This process is then trailed by eliminating the solvent under vacuum, by rotary evaporation technology, until a thin film has been prepared. After the thin-film formation, the prepared film is hydrated in an aqueous medium, above the temperature of phase transition, resulting in the development of MLV liposomes as shown in Figure 4.[9,10]

All the methods which are based on the replacement of an organic solvent by an aqueous media show that the solvents, whether miscible or immiscible with water, are substituted by an aqueous solution. First, the water-immiscible organic solution comprising lipids is injected into the aqueous phase (reverse-phase method), or the stepwise accumulation of the organic phase (specifically, ethanol) is injected into the aqueous phase (organic solvent injection method), followed by the removal of the solvent. Above said methods are capable to form liposomes with a high-encapsulation percentage of both hydrophilic and lipophilic substances. In general, the amalgamation of lipophilic drugs is achieved through their codissolution with the lipids.[10] Hydrophilic drugs are liquefied in the aqueous medium, whereas amphiphilic drugs can be liquefied in both mediums. The developments of liposome can result in the large vesicles MLV with heterogeneous size distribution; therefore, it is significant to standardize the formulation using a vesicle size decreasing method.

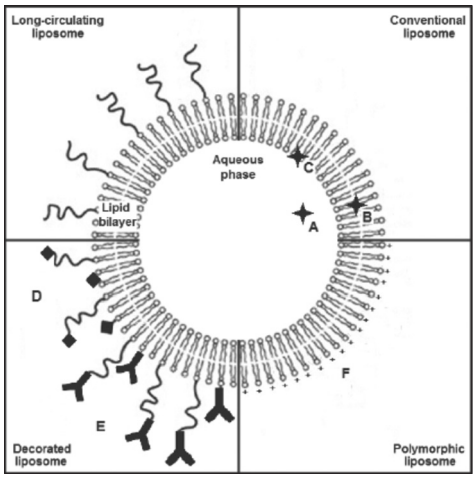

Figure 3: Composition of different types of liposomes.[8] A - Hydrophilic drug, B - Amphiphilic drug, C - The interface lipid bilayer - inner aqueous phase, D - Immunoliposomes, E - Ligands-attached liposome, F - Cationic liposomes

NOVEL TECHNOLOGIES USED FOR LIPOSOME PREPARATION

Nowadays, supercritical fluid technology, dual asymmetric centrifugation (DAC), membrane contactor technology, cross-flow filtration technology, and freeze drying technology have been employed for liposome preparation.

Liposome Preparation Methods Based on Supercritical Fluid Technology

Supercritical fluids are non-condensable fluids, which are very dense at certain temperatures and pressures beyond the critical values. As the line between the liquid and gas phase disappears, supercritical fluids have many particular characteristics compared with conventional fluids. Among these characteristics, solvents with special properties have attracted a great deal of interest from researchers. Remarkably, supercritical carbon dioxide (CO_2) is an excellent organic solvent substitute. In spite of its low cost, it is non-toxic and is not inflammable. In addition, it has a relatively low critical temperature and pressure (31°C and 73.8 bar) with the dissolution properties analogous to those of non-polar solvents.[11]

Supercritical Anti-solvent (SAS) Method

Regarding classical thin-film dispersion methods, lipids are always dispersed on the inner surface of the glass flask to

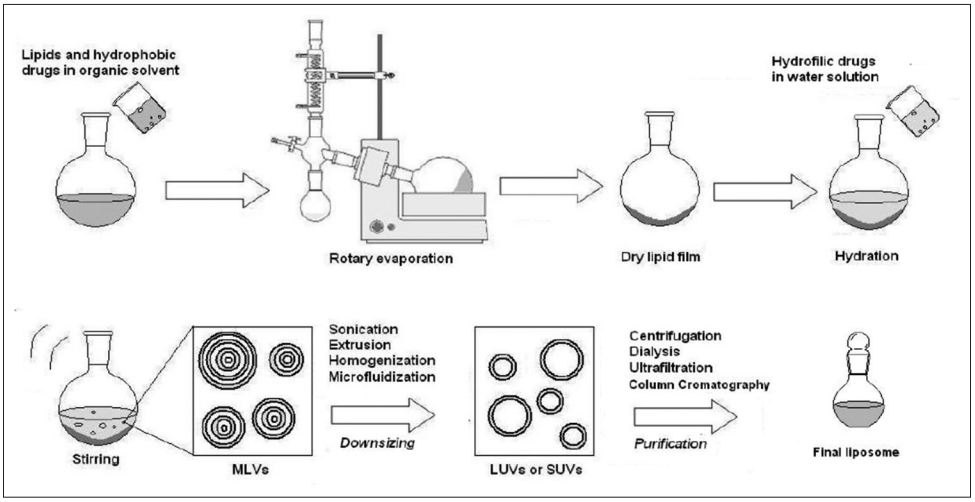

Figure 4: Production of liposome by lipid hydration, followed by vortex or stirring[9]

form a lipophilic thin film. Similarly, the SAS method is being used to achieve a fine and homogeneous dispersion of lipid materials. Briefly, in the SAS method, lipids dissolve readily in CO_2 and then precipitate in the form of ultrafine particles. The experimental procedure for SAS is shown in Figure 5a.

Phospholipid and cholesterol are dissolved in an organic solution and placed in a glass container which, together with a source of CO_2 gas, is connected to pumps linked to a precipitation vessel. Gaseous CO_2 is pumped into a high-pressure precipitation vessel by spraying through capillary tubes and then transformed into a supercritical phase because of the sudden change in temperature and pressure. Subsequently, the lipids are extracted into the supercritical phase as soon as the evaporation of the organic solvent is completely introduced, which lead to supersaturation of the solute in the CO_2 phase, and then, the lipid materials precipitate. Afterward, the organic solvent is removed by CO_2 continuously pumped into the vessel to produce fine lipid particles. Finally, liposomes are obtained by directly adding aqueous phase.[12]

Supercritical Reverse Phase Evaporation (SRPE) Method

In the conventional reverse phase evaporation method, organic solvents are usually used to dissolve lipid materials. Due to the dissolution properties of CO_2, the supercritical fluid might be an excellent substitute for organic solvents. Otake *et al.* first reported that liposomes can be prepared by a SRPE method using CO_2 as the solvent for lipids.[13] The apparatus used is shown in the Figure 5b.

Briefly, the apparatus consists of three parts: A viewing cell with a variable volume, an high-performance liquid chromatography (HPLC) pump for feeding aqueous solution into the viewing cell, and a high-pressure pump for CO_2 and pressure control by moving the piston in the viewing cell. The ethanol solution of lipid materials is measured with an electronic balance and fed into the cell. After the lipid materials are placed in the viewing cell, the cell is sealed, a magnetic tip is used for stirring inside the viewing cell, and gaseous CO_2 is introduced into the cell. The temperature is then raised to a chosen value, which could reach both the phase transition temperature of the phospholipids and the supercritical temperature of carbon dioxide. The pressure is also kept above the supercritical value. After several seconds to reach equilibration, an aqueous solution of the model drug is slowly introduced into the cell through the HPLC pump, until a sufficient amount of solution is reached. Finally, the pressure is reduced to release CO_2, and liposomal dispersion is formed.

Otake *et al.* developed the SRPE method in which they put an aqueous phase together with the solid lipid materials into a sealed viewing cell. The temperature and pressure were adjusted to suitable values, and then, the CO_2 was introduced. After equilibration, CO_2 escapes and liposomes are formed.[14] The liposomes prepared by the improved SRPE (ISRPE) method have an enhanced entrapment compared with those obtained by SRPE. Furthermore, the process is shorter because of a reduction in the apparatus required. In addition, the ISRPE method does not require any organic solvents and thus completely avoids any problems caused by organic residues which are very significant. However, whether the former or the latter procedure is used, liposomes prepared by SRPE do not have a narrow particle size distribution. Hence, post-procedures are needed to optimize this excellent method.

DAC

DAC is a special kind of centrifugation. Vials in DAC turn around the main rotation axis at a defined distance and speed, which is similar to that used in conventional centrifugation. However, the main difference between DAC and normal centrifugation is that the vials in the DAC process also turn around their own center (vertical axis) while they still turn around the center of the centrifuge as in the normal centrifugation process. In other words, the vial makes two movements in the DAC process. Interestingly, this results in two overlaying movements of the sample material in the centrifugation vial. The main rotation pushes the sample material in an outward direction in response to centrifugal forces, while the rotation of the centrifugation

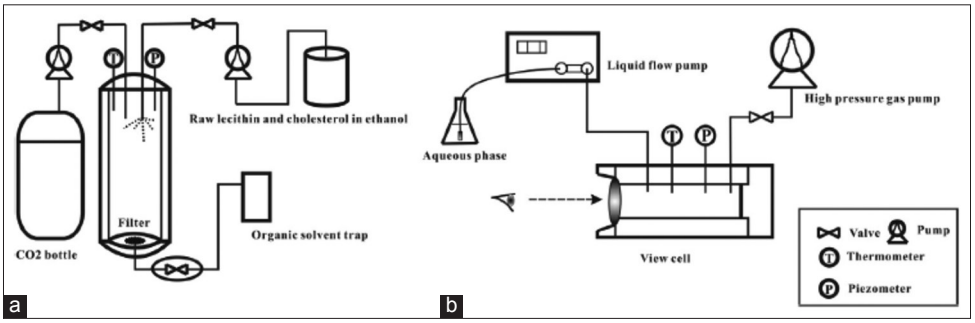

Figure 5: Experimental setup for supercritical fluid technology-based liposome preparation methods (a) the supercritical anti-solvent method and (b) the supercritical reverse phase evaporation method[13]

vial around its own center pushes the sample material in the opposite direction due to adhesion between the sample material and the rotating vial. If there is sufficient adhesion of the sample material to the vial material and the sample material is viscous enough, the latter movement, the inward transport of the sample material, is effective. Both of these factors may significantly affect the transference of energy into the sample material.[15] Therefore, DAC is particularly appropriate for the homogenization of viscous materials. Vesicular phospholipid gel (VPG) is a highly-concentrated lipid dispersion system, which can be diluted by a suitable aqueous phase and form liposomes. Since lipids are usually viscous materials, VPG is sufficiently viscous. Hence, DAC can be used for the preparation of VPG and liposomes as shown in Figure 6.

Massing *et al.* have carried out the homogenization in a dual asymmetric centrifuge in multiples of 5 min runs (since 5 min is the maximum runtime of the DAC that can be selected, the instrument is immediately restarted after every 5 min run until the total mixing time is reached) directly after weighing the constituents, namely, lipids and 0.9% w/v sodium chloride solution. After the DAC process is finished, the VPGs are redispersed in a double volume of 0.9% w/v NaCl solution to produce a liposomal dispersion. In this way, conventional and stealth liposomal preparations can be made and their mean particle sizes were 70-100 nm and 100-120 nm, respectively.[16]

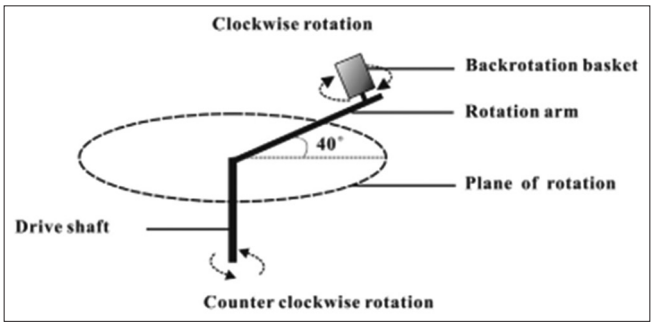

Figure 6: Schematic representation of the principle of dual asymmetric centrifuging[16]

Compared with conventional methods of liposome preparation, the DAC method has several significant advantages as follows:

1. The DAC equipment is small in size, easy to operate, and offers good reproducibility
2. Using the DAC method, liposomes with a small particle size can be produced directly without requiring other processes for granulation or homogenization
3. Water-soluble drugs have high entrapment efficiency
4. No organic solvents are used in DAC for the dispersing lipid, which is of particular significance for liposomal injections.

Liposome Preparation Methods Based on Membrane Contactor Technology

Membrane contactors can be used to mix two materials efficiently, and many kinds of dispersion systems can be prepared using a membrane contactor. It has been reported that membrane contactors have already been applied to the preparation of emulsions, precipitates, polymeric, and lipidic nanoparticles, and most recently, for the preparation of liposomes. Since homogenization of lipid and water phases is a key procedure in liposome preparation, this apparatus can be applied for preparing liposome. Based on the theoretical advantage of the ethanol injection method for liposomes, Laouini *et al.* combined a membrane contactor with a hollow fiber module. This combination could increase the membrane area and thus offers improved efficiency.[17]

As can be seen in Figure 7, the aqueous phase is poured into the module by the action of a pump, while the organic phase is placed in the pressurized vessel. The nitrogen in the bottle can push the oil phase into the system. Then, the connecting valve to the nitrogen bottle is opened, and the gas pressure is set at a fixed level. The aqueous phase is subsequently pumped through the membrane contactor module. When water reaches the inlet of the hollow fiber module, the valve connecting the pressurized vessel to the filtrate side of the membrane device is opened so that the organic phase can permeate through the pores of the hollow fibers. When

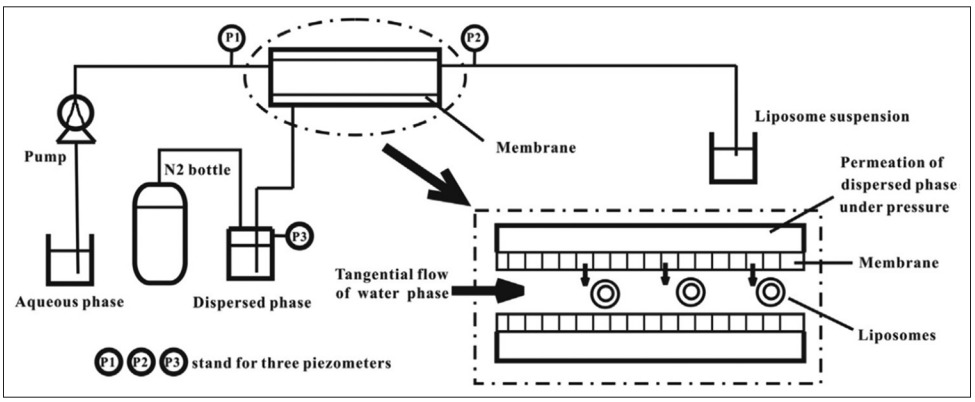

Figure 7: Schematic representation of liposome preparation based on a membrane contactor[17]

the aqueous and lipid phases meet, liposomes are formed spontaneously. The experiment is stopped when air bubbles start to appear in the tube joining the pressurized vessel to the membrane module, indicating that the pressurized vessel is empty. Then, the liposomal suspension is stabilized under magnetic stirring. Finally, the ethanol is removed by rotary evaporation to complete the final preparation. Generally, liposomes prepared using membrane contactor-based techniques have the following characteristics:

a. Homogeneous and small particle size,
b. MLV,
c. High-encapsulation efficiency for lipophilic drugs, and
d. Simplicity for scaling up.

However, if certain active drug loading methods can be introduced successfully, drugs with a high-water solubility can also undergo enhanced entrapment. This would make the technology even more attractive.

Cross-flow Filtration Detergent Depletion Method

Detergent-mediated liposome production is based on the solubilization of lipids with the aid of a suitable detergent, resulting in the formation of mixed micelles. Then, the detergent is removed, which leads to the breaking up of micelles and repacking into lipid bilayers. The edge tension increases, and finally, the curved bilayers shrink and form unilamellar vesicles.[18] However, the membranes still contain a great amount of detergent. Therefore, a technique, which combines the advantages of known detergent removal methods with an accelerated and efficient removal of detergent, can markedly reduce the preparation time and heterogeneous liposome lamellarity.

The cross-flow filtration unit consists of a starting reservoir, a pump, a filtration device (membrane system), and tubing with an integrated rotary slide valve and a manometer to monitor the retentate pressure. By increasing the retentate pressure, the pressure on the membrane increases, leading to the fast removal of detergent. The starting reservoir contains the mixed micelle solution which is subjected to tangential filtration. The filtration unit is equipped with a single membrane or membrane cassettes with a cutoff of a selected molecular weight. Using the cross-flow filtration process, liposomes of defined size, homogeneity, and high stability can be obtained. Large quantities of liposomes can be produced in a significantly shorter time compared with other methods used for detergent removal.

Freeze-drying Double Emulsion Method

Based on the traditional double emulsions method, in the freeze-drying double emulsion method, different kinds of cryoprotectants are added to the inner or outer water phase of the liposome formulation. After the formation of a W/O/W-type multiple emulsions, a sterilization process is carried out. Subsequently, liposomes in the form of lyophilized powder can be obtained through a freeze-drying procedure. Before use, the powder should be hydrated to the original volume of the water phase to be transformed into a liposome suspension. Using this procedure, the liposomes usually have a small size below 200 nm. If appropriate cryoprotectants are chosen, we can obtain a liposome preparation with a small mean particle size (approximately 100 nm), a highly efficient encapsulation (87% for calcein, 93% for flurbiprofen, and 19% for 5-fluorouracil), good reproducibility, and high stability. In addition, a sterile product can be also achieved with good storage stability by the application of freeze drying as in other drug delivery systems.[19-21]

LIPOSOME CHARACTERIZATION

The performance of liposomes in storage situations and *in vivo* is determined by specific evaluations, such as vesicle size and surface charge, composition of liposomes and membrane permeability, and quantity of entrapped drug.

Liposome bilayers are responsible for the stability; interactions with biological, such as specific tissues, cells, and proteins; and the release kinetics of the drug n liposomes. The size of the liposomes affects their *in-vivo* circulation and distribution, as this feature can regulate the amount of time that the liposomes will stay in the blood stream before being impassive. The surface charge of vesicles affects their physical stability due to the possible occurrence of fusion and/or accumulation phenomena.[3] Hence, thorough chemical, physical, and physicochemical evaluations are vital in an attempt to guarantee the efficacy and stabilization of the liposome preparation.

Chemical evaluation comprises the quantification of lipids, the characterization of lipid oxidation, and the determination of the percent drug encapsulation. As lipids signify the main constituents of the lipid bilayer, their quantification is significant in evaluating the productivity of the preparation method.[22]

Physical evaluation involves of determining the size, surface charge, and lamellarity of the liposomes. The performance and efficacy of prepared liposomes in the body and their physical stability strongly depend on the size of vesicle, and size distribution of liposomes should be determined during the manufacturing process and storage. The nature and net amount of the charge on the surface of liposome are significant parameters that impact the mechanism and extent of liposome cell interaction. The retention of the surface charge for long periods during storage subsidizes to the high physical stability of the liposome preparation.

The methods mostly used for liposome evaluation and the parameters described above are listed in Table 2.

Several liposomal preparations of anticancer drugs have also been studied in pre-clinical tumor models, and many liposomal preparations of anticancer drugs have been approved for cancer chemotherapy or are in progressive stages of clinical growth. Some of these products are listed in Table 3.

Table 2: Methods of liposomes evaluation

Evaluation	Method
Quantification of phospholipids	Bartlett method and liquid chromatography combined with Bartlett method
Lipid oxidation studies	Spectroscopy, TLC, HPLC, GC
Determination of the encapsulation percentage	Spectrophotometry, fluorescence spectroscopy, enzyme-based methods, electrochemical techniques, and HPLC
Vesicular size	Static and dynamic light scattering, microscopy techniques (light, electronic, and atomic force), size-exclusion chromatography, field-flow fractionation, and analytical centrifugation
Surface charge determination	Photon correlation spectroscopy associated with the electrophoretic mobility
Lamellarity determination	31P-NMR, electron microscopy and small-angle X-ray scattering
Lipid phase studies	X-ray diffraction, differential scanning calorimetry
Phase-transition temperature	Differential scanning calorimetry and (31PNMR or 1H-NMR)

TLC: Thin-layer chromatography, HPLC: High-performance liquid chromatography, GC: Gas chromatography, NMR: Nuclear magnetic resonance

Table 3: Liposomes encapsulated anticancer drugs

Product	Entrapped drug	Company	Therapeutic indication
Doxil Caelyx	Doxorubicin	Janssen Cilag	Kaposi's sarcoma, recurrent ovarian, multiple myeloma, and metastatic breast cancer
Myocet	Doxorubicin	Cephalon US	Metastatic breast cancer
DaunoXome	Daunorubicin	Galen-US	Kaposi's sarcoma
DepoCyt	Cytarabine	Pacira Pharma	Lymphomatous meningitis
SPI-077	Cisplatin	Sequus Pharma	Ovarian cancer
Lipoplatin	Cisplatin	Regulon	Lung cancer
Aroplatin	Bis-neodecanoate diaminocyclohexane platinum	Aronex Pharma	Colorectal, lung, and pancreatic cancer
LEP-ETU	Paclitaxel	Insys-Therapeutics	Breast, lung, ovarian cancer
EndoTAG-1	Paclitaxel	Medi-Gene	Breast, pancreatic, and hepatic cancer
ThermoDox	Doxorubicin	Celsion	Bone metastasis, breast, and hepatocellular cancer
Marqibo	Vincristine	Talon-Therapeutics	Non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and Hodgkin's lymphoma
OSI-211 (NX211)	Lurtotecan	OSI Pharma	Ovarian cancer and small cell lung cancer
LE-SN38	Irinotecan metabolite SN38	Neo-Pharm	Colorectal and lung cancer
INX-0076	Topotecan	Inex Pharm	Ovarian and small lung cancer
Alocrest	Vinorelbine	Inex Pharm	Non-small cell lung cancer and breast cancer
Oncolipin	Interleukin 2	Biomirma-USA Inc.	Kidney cancer
OSI-7904L	Thymidylate synthase Inhibitor	OSI	Colorectal cancer
CPX-351	Cytarabine and Daunorubicin	Celator-Pharmaceuticals	Acute myeloid leukemia
CPX-1	Irinotecan and Floxuridine	Celator-Pharmaceuticals	Advanced colorectal cancer

FUTURE PERSPECTIVES AND CHALLENGES

This article enlightens delivery of anticancer drugs through liposomes, which are the widely used in cancer treatments. It has been proved, based on the previous studies, that liposomes deal safety and effectiveness as compared to other conventional treatments.

The greater interest in the growth of these sophisticated drug delivery systems is to expand the efficacy and reduction in the side effects of old and new anticancer drugs. In this perspective, the optimized pharmacokinetic properties of liposomes, consequential in a better-quality toxicity profile, are still the main quarrel for the use of liposomal transporters. Other new approaches in the biology and pharmacokinetic conduct of liposomes, such as the antiangiogenic properties of cationic liposomes and the growth of immunoliposomes, also offer a great therapeutic range for these drug delivery systems. Ruenraroengsak *et al.*[23] suggest that there are many concerns about the instability of liposomes over flocculation and aggregation.

In addition, liposomes offer many targeting approaches and have shown a capable forthcoming as a new group of cancer treatment. Certain serious queries and many hindrances are quiet stay, which present definite restrictions to their complete efficacy and safety. More clinical data become presented, further understanding will lead to an additional rational design of optimized liposomes with enhanced selectivity, efficacy, and safety in the treatment of cancer.[24]

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